The production of very-long-chain PUFA biosynthesis in transgenic plants: towards a sustainable source of fish oils

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There is now considerable evidence of the importance of n-3 long-chain PUFA in human health and development. At the same time, the marine fish stocks that serve as the primary sources of these fatty acids are threatened by continued over-exploitation. Thus, there is an urgent need to provide a sustainable alternative source of the n-3 long-chain PUFA normally found in fish oils. The possibility of using transgenic plants genetically engineered to synthesise these important fatty acids has recently been demonstrated. The approaches taken to realise this outcome will be discussed, as will their prospects for providing a sustainable resource for the future.

Desaturase: Elongase: PUFA: Transgenic plants: Sustainability

There is now good evidence from many scientific publications that the human diet not only plays a vital role in normal development, but is also central to robust health; conversely, poor diet (and hence nutrition) can lead to the acquisition of, or progression to, diseased states (for recent reviews, see Go *et al*. 2003; Paoloni-Giacobino *et al*. 2003). Controlled clinical studies have provided a means by which important components of the human diet can be identified and their efficacy tested (von Schacky, 2003; GISSI-Prevenzione Investigators, 1999). In addition, the importance of genetic make-up in the responsiveness of an individual or population to any particular dietary regimen ('diet–gene' interactions) has also emerged recently (German *et al*. 2003), and has been underpinned by the completion of the human genome sequence and the parallel genotyping of large numbers of individuals. Such studies point towards considerable genetic variation amongst mankind globally. It has been estimated that there are in excess of three million polymorphisms in the human population, with these variations mainly taking the form of single nucleotide polymorphisms (Zhao *et al*. 2003). Thus, it is also important to consider these genetic components in attempts to understand and modulate human health through optimised diet and nutrition (Shastry, 2002).

An excellent example of the impact of nutrition that is of particular relevance to the topic of the present review is demonstrated by the work of Bang, Dyerberg and colleagues (Bang *et al*. 1976; Dyerberg & Bang, 1982).

These Swedish researchers have carried out pioneering studies on the prevalence of CVD in Inuit (Eskimo) communities whose diet is rich in oily fish (Bang *et al*. 1976). The resulting data led the authors to suggest the need for large-scale dietary intervention studies to determine the efficacy of high-fish-oil diets rich in n-3 long-chain (LC) PUFA (Dyerberg & Bang, 1982). It is as a result of such subsequent studies that it is now generally considered that n-3 LC PUFA are not only a pivotal component of the human diet, but they are also actively beneficial to health, providing a protective role against a range of diseases (and progressions to diseased states; for reviews, see Simopoulos, 2000; Nugent, 2004). Such human pathologies include not only CVD but also metabolic syndrome, which is a collective description for a number of indicators of risk of type 2 diabetes and obesity (Nugent, 2004). These indicators (such as high blood pressure, high plasma triacylglycerols and abnormal fasting blood glucose) are increasingly more prevalent in Western populations, and while the precise (presumptively multi-factorial) causes of these symptoms are unclear, they are of considerable concern. In particular, the apparent increases in both obesity and type 2 diabetes represent a major public health problem (Graham *et al*. 2004; Nugent, 2004). However, the observation that n-3 LC PUFA can provide some extent of pan-genotypic protection from these diseases has focused research on the biosynthesis and production of these important fatty acids (Graham *et al*.

2004). It is also noteworthy that there is now good genetic evidence (i.e. polymorphisms in biosynthetic genes) to link *n*-3 LC PUFA with a number of human diseases such as retinal dystrophy (Klaver & Allikmets, 2003).

In particular, two *n*-3 LC PUFA found in fish oils, EPA (20:5*n*-3) and DHA (22:6*n*-3), are known to be important in helping to prevent both CVD and metabolic syndrome (Graham *et al*. 2004; Nugent 2004). It has been shown that a modest intake of fatty fish may reduce mortality in men who have recovered from myocardial infarction (Burr *et al*. 1989). The GISSI-Prevenzione trial (GISSI-Prevenzione Investigators, 1999) has confirmed these findings in a large intervention study of patients surviving myocardial infarction; relatively modest (<1 g/d) consumption of *n*-3 LC PUFA markedly lowers the risk of CVD and death. Dietary EPA and DHA reduce blood coagulation, blood lipids and blood pressure and have anti-arrhythmic effects in adults. They may increase HDL-cholesterol, suppress inflammatory and allergic processes and benefit mental disorders (Hu *et al*. 2001; von Schacky 2003).

Long-chain PUFA in the human diet

The synthesis of LC PUFA is usually carried out by an aerobic process involving the sequential introduction of double bonds and C_2 elongation of a fatty acid substrate (an anaerobic process similar to the processive synthesis of polyketides has been described in several marine micro-organisms; Metz *et al*. 2001; Napier, 2002). In mammals LC PUFA biosynthesis is dependent on the dietary intake of linoleic acid (18:2*n*-6; LA) and α-linolenic acid (18:3*n*-3; ALA; hence their designation as essential fatty acids), since higher animals have lost the capacity to synthesise these two fatty acids (Wallis *et al*. 2002). Crucially, the efficiency of *n*-3 LC PUFA biosynthesis in mammals, in terms of the metabolic conversion of ALA to EPA or DHA, appears to be quite low, highlighting the desirability of supplementing this endogenous biosynthetic route with dietary LC PUFA. This metabolic conversion appears to be particularly critical at various stages of human life; for example, LC PUFA are important in the development of ocular vision in infants and may also play a role in aspects of neurological and brain development (Gil *et al*. 2003), while at the other end of the human lifespan these same LC PUFA may help alleviate a number of symptoms associated with geriatric patients (Klaver & Allikmets, 2003; Leonard *et al*. 2004).

As mentioned earlier, the primary dietary source of *n*-3 LC PUFA is fish and their associated oils. Marine fish have a similar inefficient *n*-3 LC PUFA biosynthetic pathway to that found in mammals, and also depend on the dietary acquisition of these fatty acids to supplement their endogenous synthesis (Tocher & Ghioni, 1999). However, the aquatic environment is rich in micro-organisms that synthesise (with high efficiency) *n*-3 LC PUFA such as EPA and DHA (Napier, 2002). Thus, it is through the consumption of these *n*-3 LC PUFA-rich microbes that fish accumulate (either directly or via carnivorous consumption of other fish) EPA and DHA, rather than through any innate capacity for their synthesis. This progression of *n*-3 LC PUFA up the aquatic food web has implications for attempts to supplement fish stocks by aquaculture (i.e. fish farming), not least of all because farmed fish still require dietary *n*-3 LC PUFA (Opsahl-Ferstad *et al*. 2003). Aquaculture is currently the largest consumer of fish-derived oils, and so is clearly not capable of operating in a sustainable manner. Conversely, the greater part of man's dietary fatty acid intake is in the form of plant-derived 'vegetable' oils rich in LA and ALA. Since higher plants lack the capacity to synthesise (either *n*-6 or *n*-3) LC PUFA, oils derived from them are devoid of fatty acids such as EPA and DHA. It is for this reason that vegetable oils cannot substitute for fish oils in aquaculture (Sargent & Tacon, 1999). Moreover, most plant oils are rich in *n*-6 fatty acids such as LA, rather than the *n*-3 ALA. The preponderance of vegetable oils in the modern diet has resulted in a dietary 'flood' of *n*-6 fatty acids, with a *n*-6:*n*-3 of >10:1. This situation contrasts considerably with the human diet of approximately 150 years ago, which probably reflected a ratio of 2:1, and was more likely to contain fish oils rich in *n*-3 LC PUFA (Simopoulos, 1999).

Considering all these factors, it seems that there is ample evidence for the health-beneficial properties of dietary consumption of *n*-3 LC PUFA such as EPA and DHA. Unfortunately, just as the importance of *n*-3 LC PUFA is finally being recognised by health protection agencies and the general public at large, the natural resources that provide these oils are in danger of being exhausted. For example, stocks of common marine fish in northern Europe have been over-exploited for decades, resulting in ever-reducing catch size (in terms of both numbers and maturity of fish; Opsahl-Ferstad *et al*. 2003; Graham *et al*. 2004). Thus, there is considerable pressure to identify a sustainable source of important fish-oil fatty acids such as DHA and EPA for use in the protection and improvement of human health, in addition to the requirements of aquaculture. This imminent reduction or absence of fish oils should therefore be considered a major driver for seeking and developing alternative sustainable sources of *n*-3 LC PUFA. Approaches using microbiological sources to synthesise LC PUFA for human nutrition have been developed, as exemplified by companies such as Martek Biosciences (Columbia, MD, USA), which specialises in the production of these fatty acids from natural sources such as *Crypthecodinium cohnii* (an alga rich in DHA) and *Mortierella alpina* (a fungus rich in arachidonic acid (20:4*n*-6; ARA)), by fermentation (i.e. contained culture) of these micro-organisms. However, although this approach allows a rigorous control of the growth of the LC PUFA sources, it also requires the appropriate microbiological facilities.

An alternative production system for the synthesis of LC PUFA is to use plants as 'factories' to accumulate these fatty acids in the storage lipids of their seeds (Thelen & Ohlrogge, 2002; Jaworski & Cahoon, 2003; Tucker, 2003). However, since higher plants do not accumulate these fatty acids, LC PUFA biosynthetic genes need to be introduced into a suitable oilseed crop by genetic engineering. The
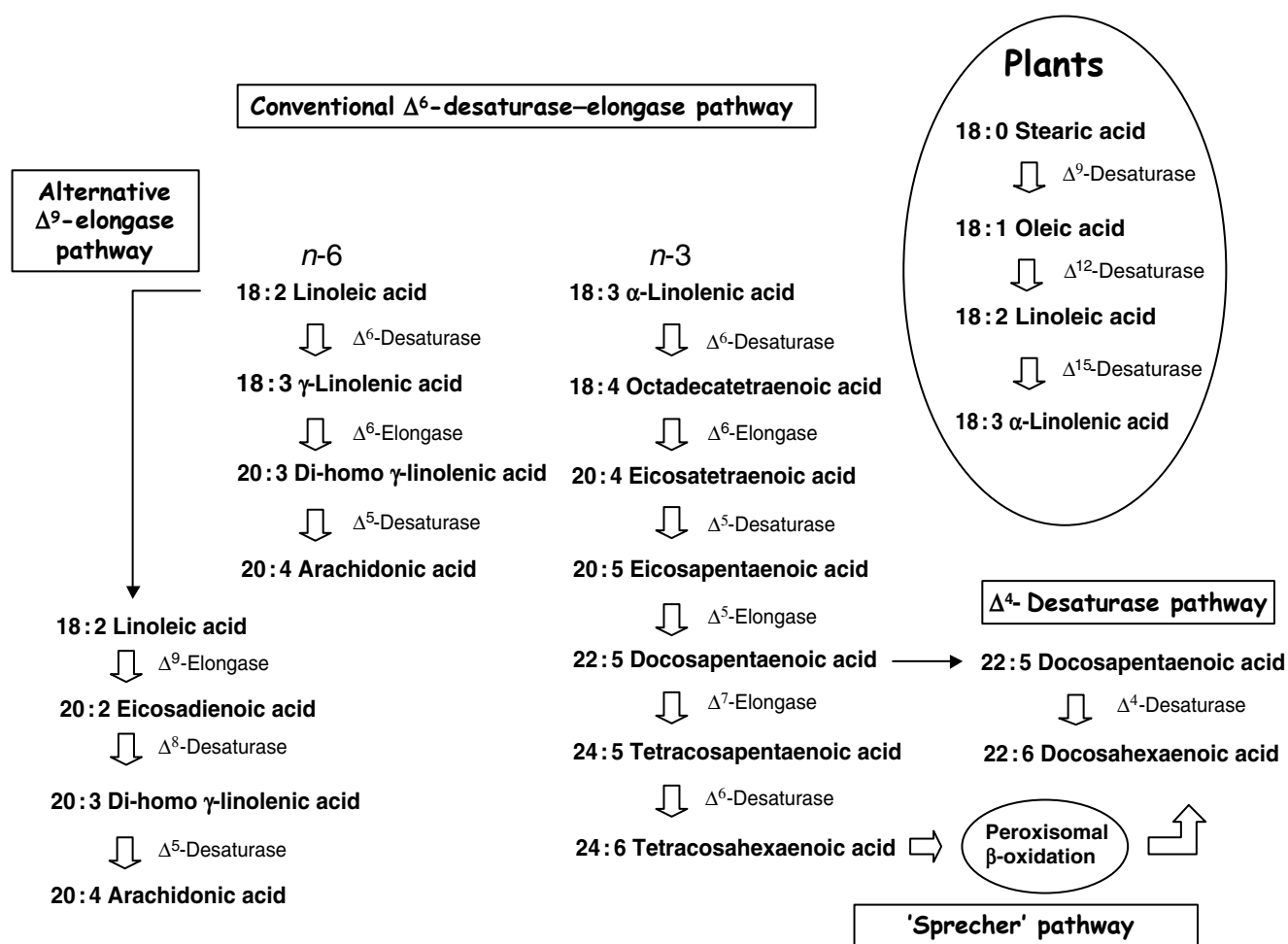
Fig. 1. Generalised representation of the biosynthesis of long-chain PUFA. The conventional Δ^6-desaturase–elongase pathway for the synthesis of arachidonic acid and EPA from the essential fatty acids linoleic acid and α-linolenic acid (synthesised in plants) is shown, as is the alternative Δ^9-elongase route (for simplicity, only the n-6 side of the alternative pathway is shown, although the n-3 substrate α-linolenic acid is also metabolised by this route). The Δ^5-elongase–Δ^4-desaturase route (prevalent in microalgae) for DHA is also indicated, as is the mammalian 'Sprecher' route that involves β-oxidation.

resultant transgenic plants would thus contain the 'trait' for LC PUFA synthesis, providing a cheap and sustainable source of these important fatty acids (Abbadi *et al*. 2001; Sayanova & Napier, 2004). Moreover, since plants are effectively C neutral, this production process is environmentally sound, utilising only solar energy and CO_2. The focus of the present paper will therefore be on recent attempts to produce transgenic plants that synthesise LC PUFA.

The metabolic engineering of transgenic plants for long-chain PUFA biosynthesis

The production of LC PUFA in transgenic plants requires the heterologous reconstitution of the biosynthetic pathway in the new host. This process in turn depends on the identification and functional characterisation of the genes encoding this biosynthetic pathway in the LC PUFA-synthesising organism, allowing the transfer of these genes to the new host. The last few years has seen considerable

progress in the identification and functional characterisation of the genes encoding LC PUFA biosynthetic enzymes (a schematic representation of the biosynthetic pathways is shown in Fig. 1), including all the microsomal fatty acid desaturases that underpin LC PUFA biosynthesis (such as the Δ^6-, Δ^5-, Δ^4- and alternative pathway Δ^8-desaturases). All these LC PUFA desaturases belong to the N-terminal cytochrome b_5 fusion superfamily, and the presence of this N-terminal electron transport domain may be associated with the 'front-end' desaturation process catalysed by these enzymes (unlike the non-fusion microsomal methyl-directed desaturases found in higher plants and some lower organisms). Examples of LC PUFA desaturases have been identified from animals, fungi, algae and some of the few plant species (e.g. Boraginaceae, Primulaceae, *Oenothera* spp.) that carry out limited PUFA desaturation. Many of these desaturases have been functionally characterised in heterologous hosts such as yeast and transgenic plants, and have been observed to function efficiently in non-native hosts (for recent reviews of progress in the characterisation

of LC PUFA desaturases, see Napier *et al.* 2003; Sperling *et al.* 2003).

The second key enzymic reaction in the synthesis of LC PUFA is microsomal acyl-elongation, in which a C_{18} PUFA substrate (usually containing a 'front-end' Δ^6-desaturation) is C_2-elongated to yield the C_{20} LC PUFA (Fig. 1). Progress on the identification of this microsomal 'elongase' has been hindered by the fact that this elongation process actually comprises four distinct and sequential enzymes, i.e. condensation (of malonyl-CoA and the PUFA acyl-CoA), β-ketoreduction, dehydration and enoyl reduction. Thus, the question arose as to whether all four components of the microsomal elongase would be required to reconstitute the elongating activity. However, open reading frames from *Caenorhabditis elegans* (a PUFA-synthesising nematode) and *M. alpina* have been identified by 'gain-of-function' (i.e. acquisition of the ability to elongate C_{18} PUFA) experiments in yeast (Beaudoin *et al.* 2000; Parker-Barnes *et al.* 2000). The identified nematode and fungal open reading frames show homology to the yeast elongation (termed ELO) genes, which are required for the synthesis of (saturated) LC fatty acids found in sphingolipids. Perhaps one of the more surprising observations in the identification of the ELO-like PUFA-elongating activities has been that the expression of these individual open reading frames is still able to reconstitute a PUFA-specific elongase; there is no requirement for the co-expression of any other components of the elongase. This finding is likely to indicate that the initial condensing enzyme (in the form of the ELO-like open reading frames) confers specificity on the elongase, with the other three reactions acting in a non-specific manner. Thus, these data indicate the possibility of re-directing or 'hijacking' an endogenous non-PUFA elongase by the expression of a heterologous PUFA-condensing enzyme and hence, the possibility of transgenic plants engineered to synthesise LC PUFA.

It is also important to consider the various biochemical nuances that exist in LC PUFA biosynthesis, as a number of 'variations on a theme' exist in nature; this topic has been the subject of several recent reviews (Drexler *et al.* 2003; Sayanova & Napier, 2004) and will therefore only be summarised here. The predominant LC PUFA biosynthetic pathway has the C_{18} Δ^6-desaturase as the first key committed step, producing γ-linoleic acid (18:3*n*-6) and stearidonic acid (18:4*n*-3) from LA and ALA respectively. These C_{18} Δ^6-desaturated products then undergo a C_2 elongation (catalysed by the Δ^6-elongase) to yield C_{20} substrates for subsequent Δ^5-desaturation to generate ARA and EPA (Fig. 1). Further elongation and desaturation can then produce DHA by two distinct pathways; either via Δ^4-desaturation of a C_{22} elongation product, or the more complicated 'Sprecher' route involving peroxisomal β-oxidation (Qui, 2003). It appears that whilst the latter route is prevalent in animals, the simpler Δ^4-desaturation pathway is present in aquatic micro-organisms. One additional important variation in the synthesis of ARA and EPA is observed in some organisms, in which an 'alternative' pathway appears to operate. Here, the order of the initial desaturation and elongation steps are reversed, with a C_{18} Δ^9-specific elongase first synthesising

C_{20} PUFA, which then undergo Δ^8-desaturation (Qi *et al.* 2002). The resulting products are then subject to conventional Δ^5-desaturation to yield ARA and EPA (Fig. 1). As mentioned earlier, all the genes encoding enzymes of the conventional and alternative ARA–EPA pathway have been cloned, as have the genes for the Δ^4-desaturation pathway for DHA synthesis.

Initial attempts to reconstitute the LC PUFA biosynthetic pathway in a heterologous host have utilised yeast as a simple model system. Beaudoin *et al.* (2000) have co-expressed the *C. elegans* C_{18}- Δ^6-elongating activity, polyunsaturated elongating activity-1, with the borage (*Borago officinalis*) Δ^6-desaturase and the *M. alpina* Δ^5-desaturase. This approach has resulted in low but marked levels of ARA or EPA in the transgenic yeast, depending on the exogenous substrate (*n*-6 *v.* *n*-3). Similar data have been obtained by co-expressing desaturases from the diatom *Phaeodactylum triconutum* with a moss Δ^6-elongase (Domergue *et al.* 2002). These authors have noted that as well as a relatively inefficient reconstitution of LC PUFA synthesis, there are a number of so-called 'side-reactions' (i.e. unexpected desaturation or elongation products). More recently, a very detailed analysis of the biochemical steps involved in heterologous LC PUFA biosynthesis in yeast has been carried out, revealing a number of potential bottlenecks in the conventional Δ^6-desaturase–elongase pathway (Domergue *et al.* 2003). This analysis has indicated that one of the major constraints in this pathway may be the different substrate requirements for desaturases or elongases; most non-animal microsomal PUFA desaturases use glycerolipid-linked substrates (i.e. fatty acids esterified to membrane lipids such as phosphatidylcholine), but fatty acid elongation requires acyl-CoA substrates. Thus, there is a requirement for acyl-exchange between glycerolipids and the acyl-CoA pool; this reaction itself is mediated by an enzyme(s) that may display selectivity towards its substrates (Beaudoin & Napier, 2004).

First successful production of C_{20} long-chain PUFA in transgenic plants

Two studies on the heterologous synthesis of LC PUFA in plants have recently demonstrated the possibility of using transgenic plants to synthesise these important fatty acids. The first study utilised the alternative Δ^9-elongase pathway, after the serendipitous isolation of a C_{18} Δ^9-elongating activity from the marine microalga *Isochrysis galbana* (Qi *et al.* 2002). On the other hand Wallis & Browse (1999) have identified the specific Δ^8-desaturase activity associated with this pathway from *Euglena*. The presence of the alternative pathway in *Isochrysis* was unexpected and, because such a Δ^9-elongating activity would utilise endogenous LA and ALA as substrates (in the form of CoA), expression of this *Isochrysis* gene in transgenic plants has provided an opportunity to assess the function of a heterologous PUFA elongase alone.

Expression of the *Isochrysis* Δ^9-elongase in transgenic arabidopsis, using the constitutive CMV 35S promoter, has resulted in comparatively high levels (approximately 15%

total fatty acids) of the C_{20} PUFA eicosadienoic acid (20:2n-6) and eicosatrienoic acid (20:3n-3), through the elongation of LA and ALA, depending on the tissue type (Qi *et al.* 2004). However, values for n-6:n-3 elongation products do not exactly mirror the total levels of endogenous substrates LA and ALA, which probably reflects the extent of acyl exchange between the microsomal 'eukaryotic' and the plastidic 'prokaryotic' pathways in different tissue types (for a full discussion of these data, see Napier *et al.* 2004). Interestingly, the accumulation of C_{20} di- and trienoic fatty acids does not cause any perturbation to the morphology or viability of the transgenic arabidopsis plants, which is in contrast with the ectopic expression of the seed-specific fatty acid elongase 1 elongating activity. This latter enzyme directs the elongation of oleic acid to 20:1 (n-9) and 22:1 (n-9) and, in transgenic arabidopsis plants accumulating >10% C_{20-22} monounsaturates, results in dramatic morphological alterations including perturbed plastid membranes (Millar *et al.* 1998).

To fully reconstitute the alternative C_{20} PUFA biosynthetic pathway, transgenic arabidopsis lines expressing the *Isochrysis* Δ^9-elongase have been additionally sequentially transformed with the *Euglena* C_{20} Δ^8-desaturase and the *M. alpina* Δ^5-desaturase. This process results in the conversion of the elongation products 20:2 (n-6) and 20:3 (n-3) to 20:3 (n-6) and 20:4 (n-3) via Δ^8-desaturation, and subsequent Δ^5-desaturation of these products to ARA and EPA respectively (Qi *et al.* 2004). These two LC PUFA accumulate in leaf tissues of transgenic arabidopsis plants to a combined level of approximately 10% total fatty acids, the majority being ARA (n-6). Again, this outcome does not reflect the levels of n-6:n-3 substrates (predominantly ALA (n-3) in vegetative tissue). In addition to accumulation of ARA and EPA, several other C_{20} PUFA were also detected and identified as sciadonic acid (20:3 $\Delta^{5,11,14}$) and juniperonic acid (20:4 $\Delta^{5,11,14,17}$). These two PUFA are non-methylene-interrupted and appear to have been synthesised as a result of the 'promiscuous' activity of the Δ^5-desaturase. Whether this outcome represents a perturbation of substrate channelling in the reconstituted alternative LC PUFA biosynthetic pathway remains to be determined, but it is worth noting that the enzyme used in this study (the *M. alpina* Δ^5-desaturase) has previously been observed to synthesise unusual Δ^5-desaturated C_{18} fatty acids when individually expressed in transgenic rapeseed (Knutzon *et al.* 1998). Whilst sciadonic and juniperonic acids were not primary targets for the synthesis and accumulation in transgenic plants, recent evidence suggests that these LC PUFA may also be beneficial to health and may play a role in modulating some aspects of human metabolism. In particular, 2-monoacylglycerol containing sciadonic acid has been observed to act as a ligand for the human CB_1 cannabinoid receptor (Nakane *et al.* 2000). Both sciadonic and juniperonic acids are found in a number of species of pine seeds, and as such have been consumed by human subjects without demonstrating any anti-nutritional effects (Berger *et al.* 2002).

The use of the alternative Δ^9-elongase–Δ^8-desaturase to successfully reconstitute LC PUFA biosynthesis in

transgenic plants has been recognised as an important breakthrough in the production of these compounds in a sustainable manner (Green, 2004; Napier *et al.* 2004). However, whilst these current data represent a 'proof-of-concept' demonstration, the results of seed-specific expression of this pathway in a transgenic oilseed crop are still eagerly awaited.

Thus, the studies of Abbadi *et al.* (2004) on the expression of the Δ^6-desaturase–elongase pathway provide some further insights into the feasibility and constraints on LC PUFA synthesis in transgenic oilseeds. Using enzyme activities from a number of different PUFA-accumulating species, transgenic linseed and tobacco lines have been generated that express the Δ^6-desaturase, the Δ^6-elongase and the Δ^5-desaturase. These three activities have been placed under the transcriptional regulation of seed-specific promoters and introduced into the transgenic plant as a single integration event. Analysis of the seeds of transgenic tobacco or linseed has revealed very high levels of Δ^6-desaturated fatty acids yet only relatively low amounts of ARA and EPA. Whilst these data clearly demonstrate the reconstitution of the 'conventional' PUFA biosynthetic pathway in transgenic seeds, they also parallel earlier observations in yeast on the efficient synthesis of C_{20} PUFA (Beaudoin *et al.* 2000; Domergue *et al.* 2002, 2003). Further detailed analysis of transgenic linseed expressing these activities has revealed a number of subtle observations (Abbadi *et al.* 2004). First, although the Δ^6-desaturase and the Δ^6-elongase appear to function at very different rates, the two transgenes are transcribed at similar levels. Second, although the Δ^6-desaturated substrates for elongation accumulate at high levels in the microsomal membranes, particularly at the *sn*-2 position of phosphatidylcholine, this outcome is not reflected by a concomitant increase in the Δ^6-desaturated acyl-CoA; thus, there is inefficient exchange from phosphatidylcholine into the acyl-CoA pool. As an additional (and converse) complication, C_{18} n-3 fatty acids appear to be very efficiently channelled from phosphatidylcholine into triacylglycerols (most probably via the action of an acyl-CoA-independent acyltransferase activity), by-passing the site of elongation and effectively precluding them from further modification (Abbadi *et al.* 2004; Beaudoin & Napier, 2004; Napier *et al.* 2004).

Taking these observations together, it seems likely that a major constraint on the synthesis of LC PUFA via the conventional Δ^6-desaturase–elongase route is the dichotomy of substrate requirements exhibited by the glycerolipids desaturases and the acyl-CoA microsomal elongase. However, the levels obtained of ARA and EPA in the seeds of transgenic linseed are still reasonable, even allowing for the clearly suboptimal exchange and channelling of acyl substrates. Thus, these results should be taken as highly encouraging for the successful synthesis of LC PUFA via this pathway.

Future prospects: towards the production of DHA

The accumulation of C_{20} LC PUFA ARA and EPA in transgenic plants expressing either the conventional or alternative desaturase–elongase pathways is a major

achievement, and whilst the levels are relatively modest (approximately 3% EPA), they still represent levels that could provide nutritional enhancement to animal diets. However, an additional target for the alternative sustainable production of 'fish oils' in transgenic oilseeds is the synthesis and accumulation of DHA. The simplest route for DHA synthesis is by the C_2 elongation of EPA, via the action of a Δ^5-elongase (to elongate EPA to docosapentaenoic acid ($22:5n$-3)) and subsequent desaturation with the C_{22} Δ^4-desaturase (Qiu, 2003). Thus, two additional transgenes need to be introduced into transgenic plants that already accumulate EPA. The cytochrome b_5-fusion C_{22} Δ^4-desaturase has been identified and functionally characterised from several aquatic micro-organisms (Qiu *et al*. 2001). More recently, the specific elongating activity required for the synthesis of $22:5$ (n-3) from EPA has been characterised (Meyer *et al*. 2004; Pereira *et al*. 2004). However, it might be expected that the previously-mentioned constraints on efficient elongation associated with poor acyl exchange between glycerolipids and the acyl-CoA pool might also apply to the synthesis of DHA, with a potential bottleneck occurring via the accumulation of EPA in phosphatidylcholine (see Fig. 1). As also noted earlier, the expression of the LC PUFA biosynthetic pathway in transgenic plants results in the synthesis of both n-6 and n-3 products, rather than just the predominantly n-3 LC PUFA found in fish oils. Thus, more efficient channelling (or re-routing) of both substrates and products will be required to optimise the heterologous reconstitution of n-3 LC PUFA synthesis in plants. Thus, it will be of considerable interest to assess the possibility of using the non-aerobic polyketide synthase-like system to synthesise DHA in transgenic plants, since this biosynthetic route does not apparently require acyl-exchange (at least during the processive synthesis of DHA; Metz *et al*. 2001; Napier, 2002).

Conclusions

The possibility of 'reverse engineering' LC PUFA biosynthesis into transgenic plants has now been successfully demonstrated for ARA and EPA. These important breakthroughs have also provided new insights into the maintenance of lipid homeostasis in plants, and the key role of acyl-exchange enzymes in LC PUFA reconstitution. This additional knowledge will underpin future attempts to engineer the synthesis of C_{22} LC PUFA such as DHA. It is therefore to be hoped that the use of transgenic plants as a source of fish oils for human health and nutrition can be considered as a sustainable alternative to the diminishing marine fish stocks (Graham *et al*. 2004). It should also be remembered that since aquaculture is also dependent on fish oils for the correct nutrition of farmed fish species, transgenic-derived plant oils enhanced by the presence of LC PUFA may also be able to sustainably replace this additional demand (Opsahl-Ferstad *et al*. 2003).

Acknowledgements

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